

KARYOLOGICAL STUDIES AND CHROMOSOME NUMBERS IN *HYPARRHENIA AUCTA* AND *H. HIRTA*.

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(With Plates XIV, XV.)

INTRODUCTION.

The area of distribution of *Hyparrhenia hirta* is extremely extended. It covers the whole continent of Africa, especially its eastern side, with only a few breaks. It passes even to Western Asia. In the Union it was recognised quite long ago as an important species for soil conservation. However, the use of this grass in eroded areas has been restricted because of the low germinability of its seeds.

Plant ecologists and grass breeders found that the above species includes two or three forms without sharp and distinct differences amongst them.

The present paper deals mostly with these two problems. Its aim is to find the causes of the partial sterility in *Hyparrhenia hirta* and reveal the karyological differences, if any are present, among the morphological forms.

TECHNIQUE.

In order to have material for cytological investigations one first must get seeds for germination.

In the beginning of this work soaking of collected seeds in 95 per cent Alc. was often used in order to separate easily empty scales and florets from fertile seeds. To check the influence of this treatment on root mitosis and on the germination of seeds, a small series of experiments was carried out with different times of treatment compared with untreated control samples of the same samples of seeds. It was found that 5' or even 10' treatment of florets with 95 per cent Alc. had no influence on the root mitosis, but definitely affected the germination. Thus to be on the safe side when this treatment was used, the material was soaked for 5' and afterwards the florets were transferred to water for 15'—30'. Subsequently fertile seeds were selected under a dissecting microscope using needles.

To obtain adventitious roots from mature plants dug from the soil the following method was used. The leaves and stems were cut to a height

of 10—15 cm. above soil level and smaller roots were also cut. The plants were then soaked overnight or for 24 hours in a Hortomone solution of the concentration: $1\frac{1}{2}$ cc. of Hortomone to 1 pint of water. Next the plants were placed between sterilised wet blotting paper in sterilised dishes and the dishes then placed in a thermostat at -40°C . On the tenth day usually the first adventitious roots appeared. It was found that the treatment with Hortomone did not affect the mitosis. Shorter soaking, for example for 4 hours also had no effect on germination, but stronger concentrations of Hortomone gave less satisfactory results.

Often plants were potted out in soil after such treatment and gave a good crop of young roots in 1—2 weeks' time. Most of the root tips for microtome technique were fixed mainly in two fixatives:

I. Navashin fixative, Stockholm modification (see Maheshwari, 22).

Liquid A: Chrom. acid—1 gm., glac. Acet. ac.—10 cc., water—65 cc.

Liquid B: Commercial formalin—40 cc., water—35 cc. Before use equal volumes of A and B are mixed.

II. Levitsky fixative (Ardulov, 2).

The best results were obtained with a modification of the above fixative. Equal parts of the following solutions were mixed: (a) 4 per cent formal; (b) 2 per cent Chrom. acid. The material was infiltrated for 15' with this mixture, using a pump.

For staining microtomed material Newton's Crystal Violet stain was used after Navashin's fixative. Adventitious root sections were stained for 30' and differentiated in Lugol for 30". Primary root sections were stained for 20' and differentiated in Lugol for 35". These times of staining were the best to bring out chromosome morphology, but for counting deeper staining was preferred, and the time required for differentiation was shorter—20". Another modification of value for morphology was: staining 15', Lugol 30—32". For the above staining techniques the most satisfactory thickness of sections was 10 mu. After the treatment with Levitsky fixative the material was stained with Iron Hematoxylin. Before staining the mordants used were: (a) three days' treatment in 1 per cent Chrom. acid followed by short washing in distilled water and transferring to (b) 6 per cent alum solution for 24 hours. The thickness of sections here was 7—8 mu.

FEULGEN METHOD. MODIFIED:

After the use of Navashin fixative the Feulgen method of Warmke's (45) modification for squashing of isolated sections and isolated cells with metaphase was used. Slides with root tip sections (thickness 8 mu.) were hydrolysed in N HCl for 45' at 60°C . Next followed staining in

leucobasic fuchsin for 3 hours, then—mounting in dilute balsam. Squashing of the sections was advisable only on the second to third day after mounting. With this method it was possible to squash sections and to spread the chromosomes in 3 slides of *H. hirta* (7.VII.48—11.X.48). The counting was $2n = 44$. This squashing method was a difficult and laborious process. It was noted that: (a) squashing was impossible on the sixth or seventh day after mounting, and (b) that in a few months' time the chromosomes lost their colour.

SQUASH METHODS:

There are some difficulties in the staining of pollen grain mother cells which are not easy to explain. The best results were achieved in the following procedure with the anthers:

1. fixation in 1 p. of glacial acetic acid and 5 p. of absolute alcohol.
2. 3 days in aceto-carmin.
3. mounting in 45 per cent acetic acid, with slight heating, followed by squashing and immediate mounting in Euparal.

With direct squashing in aceto-carmin it was difficult to obtain good spreading as well as good staining. It was helpful to use aceto-carmin mixed with 4 per cent Iron Acetate as follows: 1 drop of Iron Acetate added to 10 cc. of aceto-carmin. The mixture must be prepared fresh nearly every day.

Sometimes good results were obtained by using carmin saturated in glacial acetic acid instead of solution 1.

SNAIL STOMACH JUICE (Fabergé, 10):

The sections prepared from fresh root tips were fixed (acetic alcohol) and then passed to 70 per cent alc. Next, the sections were passed through alcohol and water changes and left in snail stomach juice for $5\frac{1}{2}$ hours at $\pm 40^{\circ}\text{C}$. Afterwards sections were washed in water, mounted in a drop of aceto-orcin, heated and squashed. They had been left in aceto-orcin for 20'. Sometimes it is necessary to use an additional 1—4" of heating during the staining.

The active substance here is probably cytase and the best source of it is the edible snail—*Helix pomatia*. We used the South African relatives of this genus with very good results. The process of dissection of the snails and of extraction of juice is, however, troublesome and time devouring.

PECTINOL METHOD (MacKey and Clarke, 21):

Pectinol in 1 per cent watery solution may be used at room temperature or at temperatures not exceeding 49°C . Entire roots may be treated or

sections of root tips previously fixed, passed through the alcohols and washed carefully in water. Then the above sections were transferred to 1 per cent pectinol for 5—6 hours at 40°C., or for 15—17 hours at room temperature. Finally they were washed in water, mounted in a drop of aceto-orcein (or aceto-carmine), squashed and stained.

The roots preserved for a long time in 70 per cent alcohol require more complicated treatment as follows: 1. prepare hand sections. 2. transfer for 1—1½ hours to the Warmke liquid (equal parts of N HCl and 95 per cent alcohol) at 58°—60°C., 3. pass sections quickly through 95 per cent alcohol, and then gradually bring down to water, 4. change water. 5. leave sections in 1 per cent pectinol for 24 hours at room temperature, 6. pass through water, squash and stain with aceto-orcein.

GERSTEL METHOD (12):

The original Gerstel method, when properly modified for difficult grass roots, gave quite good results with regard to counting of the chromosomes. Here again entire root tips or sections can be treated. With *Hyparrhenia hirta* it was found preferable to prepare sections from the fresh living roots which were immediately fixed. The procedure was as follows: 1. fixation in cold 10 per cent N HCl and infiltration for 15', 2. transfer sections in the 10 per cent N HCl to the thermostat at 58°C. (or 60°C.), for 1 hour and 30 minutes, 3. 95 per cent alcohol for ½—1 minute and transfer to aceto-orcein mixed with HCl (in the proportion of 1 cc. N HCl + 10 cc. aceto-orcein), 4. aceto-orcein + N HCl for 1 hour, 5. transfer to a drop of pure aceto-orcein, heat and squash. This procedure was found to be necessary for difficult roots like those of *H. hirta*. Applied to other plants, the procedure may be simplified when, for example, the transfer to 95 per cent alcohol may be omitted, aceto-orcein may be used without HCl, and the times may be shortened.

O'MARA METHOD (32):

This method gives very distinct staining of resting nuclei (chromocenters) and prophase stages. Metaphase chromosomes were, however, rather difficult to stain properly. Fresh root tips or sections of them are treated as follows: 1. 3 per cent methanol for 3 hours, which is then replaced by the following fixative: methanol—65 parts; chloroform—5 parts; glacial acetic acid—30 parts; 2. leave in the above fixative for 13 days (other plants require only 3 days, e.g. *Kniphofia* roots); 3. transfer to a drop of aceto-orcein (which may be used with N HCl as described under Gerstel's method) or to fresh aceto-lakmoid; 4. squash and heat at least twice; 5. stain 5—10'.

LEUCOBASIC FUCHSIN SQUASH METHOD:

Root tips are fixed in acetic alcohol for not less than 2 hours. Two modifications were used:

A. Sections of the fixed material were treated as follows:

1. In 1 per cent pectinol for 4 hours at 40°C.; 2. quick washing in water; 3. hydrolysis in N HCl at 60°C. for 1 hour; 4. long staining in LB-fuchsin (up to 12 hours); 5. squashing in a drop of LB-fuchsin.

B. Sections were treated as follows:

1. In 4 per cent NH_4OH at 60°C. for 15'; 2. transferred to N HCl for 1½ hours at 58°C. or 60°C.; 3. long staining in LB-fuchsin (from 3—12 hours); 4. squashing in a drop of LB-fuchsin (or a drop of 45 per cent acetic acid).

Many modifications of above squash methods are useful only for counting the chromosomes. One must always remember that aceto-orcein causes much more swelling of the chromosomes than aceto-carmine. With the LB-fuchsin squash method chromosomes are not swollen but they stain rather faintly. For the morphology of chromosomes in *Hyparrhenia hirta* the squash technique is useless.

The chief aim of this chapter is to save grass cytologists much time and trouble as it is the writer's hope that his experience will be useful for future grass investigations in South Africa.

MEIOTIC CHROMOSOMES, NUMBERS AND BEHAVIOUR.

The investigation of meiotic chromosomes (bivalents) in both 1-meiotic metaphase and in diakinesis was done mainly in two ways: (a) with variations of the squash method and (b) with material fixed in Navashin liquid and Carnoy and sectioned on the microtome. The details of the techniques and difficulties met with have been described in the previous chapter. The results will now be given. From the commencement of this work with *Hyparrhenia hirta* (strain $2n = 44$) in early 1944, it was clear that the plant is extremely variable in number of chromosomes in pollen grain mother cells and rich in many irregularities during disjunction. The species always gives difficulties in squashing and staining with aceto-carmine and with other aceto-methods. It was first thought that the variable numbers in the haploid set might have been due perhaps to very hot and dry weather in April, May and June, 1944, but when material was collected in a wet season in the following year the results were the same. It was soon found that observations on the valents association in diakinesis are very uncertain due to the smallness and indistinctness of bivalents.



TEXT FIGURE 1.

- FIG. 1.—*H. aucta*. Root tip metaphase with $2n = 20$. Fixation—Levitsky (5:5) with 4% formol, 1% chrom. acid 3 days. Stained—iron haematox. Thickness 7μ . B. & L. Fl. oil immers. $\times 98$ and Comp. oc. $\times 10$. Magnification $\pm 1400 \times$.
- FIG. 2 TO FIG. 5.—Drawn with B. & L. Fl. oil immers. $\times 98$ and comp. ok. $\times 15$. Magnification ± 2800 .
- FIG. 2.—*H. hirta*, root tip metaphase with $2n = 44$. Fix.—Levitsky (5:5) with 10% formol, 1% Ac. Chrom.—3 days. Stained—iron haematox. Thickness 7μ .
- FIG. 3.—*H. hirta* from U.S.A., adv. root tip metaphase with $2n = 30$. Fix.—Navashin. Staining—Crystal Violet. "S"-like chromosome present.
- FIG. 4.—*H. hirta* from U.S.A. $2n = 30$. All conditions as above. "S"-like chromosome absent.
- FIG. 5.—*Schizochyrium semibarbe* from Frankenwald (from the field numbered in 1945—P4). Fix.—Levitsky (5:5) with 10% formol. Stained—iron haematox. Thickness 8μ . Root tip metaphase, $2n = 50$.
- FIG. 6.—Optics as Fig. 7. Tubes 0. Decreased-magnification $\pm 750 \times$. Stained as Fig. 7. Enormously big diads. Resting nuclei. Laggars in cytoplasm still persistent.
- FIG. 7.—*H. hirta*, $2n = 44$. P.g.m.c. Acetocarmine squash. Leitz, big microscope. B. & L. oil Immers. $\times 97$, comp. ok. $\times 10$. Tubes 160. Magnification $\pm 1700 \times$. First metaphase; $n = 22$.

In December, 1945, 22 bivalents (Pl. XIV, fig. 4, Text-Fig. 1; 7) were obtained in about half the number of pollen grain mother cells investigated, both in freshly squashed and in microtomed material. It was, however, impossible to state anything definite on their morphology. The rest of the metaphases showed the following numbers: 20, 18 (Pl. XIV, fig. 5), 16 (very rare), 14 (Pl. XIV, figs. 2 and 3 give the same diakinesis taken in two levels). Even numbers were usually found in these observations and odd numbers, e.g. 21 and 17, were rarely found. From the beginning of these observations many laggars (Pl. XIV, photo. 6) were evident in

anaphases. They were found in every season, in material collected in moderately warm weather in December (Pl. XIV, fig. 8), as well as in a very hot period during February and March. It was thought important to determine exactly whether the laggings were included later in the daughter nuclei or whether they persisted in the cytoplasm during the telophase and what was the final fate of these bodies. In anaphase they were found in various positions in relation to the poles and the equator, as well as in very variable numbers. Numerous small laggings were present on the equator (Pl. XIV, fig. 6) in the beginning of anaphase. They were also present in the middle telophase (Pl. XIV, fig. 7) approaching one of the daughter nuclei, sometimes from two directions. In some cases they were arranged in one line between two daughter nuclei during late telophase (Pl. XIV, fig. 8). In squash aceto-carmin slides they could be found in different stages, for example in diads (Text-Fig. 1; 6). In tetrads they were very distinct, either like separate chromosomes (Pl. XIV, fig. 9), considerably decreased in size, or organised into dwarf nuclei (Pl. XIV, fig. 11). In very young pollen grains with 1 nucleus, before the formation of the exine, they have been seen either single (Pl. XIV, fig. 10) or even as many as four (Pl. XIV, fig. 16).

This was the last stage in which they were observed and in older pollen grains they are not present.

Probably they are later absorbed completely by the cytoplasm as they are never found subsequent to the 2-nucleated stage in pollen grain development.

Nevertheless, the accumulated evidence shows clearly that abundant lagging can be the cause of variable and small chromosome numbers in first meiotic metaphase. Persistence of the laggings through all the stages of pollen grain development including 1-nucleated pollen grains shows that deficient chromosome numbers can not be readjusted and that they are present in pollen grain nuclei as well.

In the light of the above facts we can consider the lagging of chromosomes in the first meiotic metaphase as a chief cause of deficient chromosome numbers in male gametes and as a chief cause of partial sterility of seeds in this strain of *Hyparrhenia hirta*.

MATURE POLLEN GRAINS AND THEIR GERMINATION.

The first attempt to investigate mature pollen grains of *Hyparrhenia hirta* (strain 2n = 44) showed that the task would not be easy. From the beginning of this work in 1944 and 1945 a high percentage of degenerated and shrunken pollen grains were found; these had an extremely distorted abnormal shape and in many cases it was clear that they were empty, containing neither cytoplasm nor nuclei. The same has been recently

observed in *Anthoxanthum* by Lima de Faria (20), who considered it to be an indication of a high degree of disturbance in meiosis and a sign of extreme degeneration. It was estimated that in 1944 and 1945 there were approximately 20 per cent of degenerated pollen grains in each anther. An attempt was made to germinate the pollen grains using different concentrations of saccharose with agar and gelatine according to H. E. Newcomer's prescription (31). Different types of water were used in the preparation of the media: (a) distilled water; (b) Johannesburg Municipality tap water and (c) well water from Frankenwald. Keeping in mind Brink's observation (4) that $\text{pH} = 7$ is optimal for the germination of pollen grains, the pH of Johannesburg tap water was determined, and was found to be 8.0. Accordingly some of the media were acidified slightly but no success was obtained.

Swelling of pollen grains was observed, and sometimes they actually exploded, but usually not much development was seen. After application of a 40 per cent concentration of saccharose a few tubes were observed, but they were usually very short and soon died.

Artschwaeger and Maguire (1) have recently used the same concentration with success for pollen grains of *Sorghum vulgare*. In this species the pollen grains started to germinate 15' after sowing and the growth of the tubes continued for 3 hours. *H. hirta*, in spite of being a member of the same tribe Andropogoneae, behaved quite differently.

Nevertheless, from these unsuccessful preparations of the tubes some important observations were made, not on germinated pollen grains but on those swollen enough to give a clear picture of their nuclei after staining by Newton's crystal violet method.

The mature pollen grain is 3-nucleated, as is usual in the Gramineae (except in the case of degenerated pollen grains). Pollen grains with 4 nuclei (Pl. XIV, fig. 12) were found three times on slides stained with crystal violet and twice in temporary aceto-carmin squashes (5.6.44). Once even a 5-nucleated pollen grain was seen (Pl. XIV, figs. 14 and 15).

This matter has been given a fair amount of attention in recent literature. Thus de Mohl (24) described 4, 5, 6 and even 7-nucleated pollen grains in *Hyacinthus* plants, the bulbs of which had been accelerated in their growth. Apart from these there were some giant pollen grains which the author considered to be diploid. According to de Mohl pollen grains with supernumerary nuclei obtained their additional nuclei from the division of generative nuclei. In his opinion they are supernumerary male gametes and are fertile and compatible. The writer feels, however, that there is not sufficient evidence for this view. On the other hand, La Cour (18) in *Tradescantia bracteata*, and K. Sax in *Tradescantia virginica* (38) found pollen grains with supernumerary nuclei after special

IDI OGRAM OF HYPARRHENIA CHROMOSOMES. x 2800.

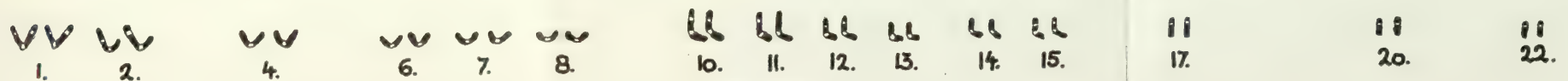
H. aucta.



H. h. 2n=44.



H. h. 2n=30.



TEXT FIGURE 2.

treatment with heat. These investigators considered they were derived from the additional mitosis of generative nuclei and supposed them to be fertile. The former author also counted the normal number of chromosomes in supernumerary nuclei ($n = 6$). Lima de Faria (20, p. 540—543) found pollen grains with supernumerary nuclei in *Anthoxanthum* and proved that they were derived from tube nuclei (except in one case in which there were two additional mitoses in both tube and generative nuclei). He considers the possibility that they are the result of degeneration.

Artschwager and Maguire (l.c., p. 664) found supernumerary nuclei in pollen grains of *Sorghum vulgare*, which they claimed were derived from generative nuclei.

K. Sax (39) in *Tradescantia* and Upcott (43) in *Belladonna* found some pollen grains with supernumerary nuclei from tube nuclei in triploid strains. They regard this occurrence as a result of unbalance in chromosome numbers in meiosis of triploids and suppose them to be infertile.

In all the five described cases of supernumerary nuclei in *H. hirta* there was no distinct evidence as to the source from which the additional nucleus of the pollen grain is derived, as mitosis has not been observed. The only evidence of their nature could be found in their different ability to take up stains as compared with normal pollen grains, as they stain like abnormal nuclei.

Summing up the observations on the mature pollen grains the two following causes of partial sterility in *H. hirta* should be added to those given:

- (a) degeneration of pollen grains;
- (b) occurrence of pollen grains with supernumerary nuclei.

In a test of the viability of pollen grains with N.R. (neutral red—A. Guilliermond) in general all except degenerated and deformed ones were found to be still alive in spite of their inability to germinate in artificial media.

The normal mature pollen grains of *H. hirta* are very rich in starch as shown by their reaction with Lugol.

CHROMOSOME MORPHOLOGY AND CHROMOSOME SET.

The following method was used to investigate the morphology of chromosomes in detail. Five to ten of the best metaphase plates in root tips were selected and drawn as exactly as possible with a Zeiss drawing apparatus and at the same magnification, approximately 2,800 x. Next the chromosome drawings were cut out with scissors and arranged in a row on a sheet of cardboard in such a way that the bases of the chromosomes were on the same level. Homologous pairs were arranged according

to their heights in descending order so that the longest pair in each group was on the left and the shortest on the extreme right of each group. The conventional grouping was followed, namely, on the left the "V"-shaped chromosomes; next to them the "L"-shaped ones and lastly the rod-shaped pairs. In this way all homologous pairs of the same height were arranged approximately on the same vertical line. Having drawn all the best metaphase chromosomes and placed them on one sheet of paper the average diagrammatic pair was prepared from them for each vertical row of pairs.

The diploid set of such diagrammatic pairs forms an idiogram of the chromosome set (a term introduced by S. Navashin (27)) and its method of use can be found in older literature (*vide* L. Delaunay's paper on *Muscari* (8) and more recently Taylor's paper on chromosomes of *Aloe*, *Gasteria*, *Haworthia* (41, 42) and M. Navashin on *Crepis* (28)). Having prepared idiograms for *H. aucta* and for two strains of *H. hirta* the task of comparing them became much easier (see idiograms Text figure 2.) The diploid set of chromosomes in *H. aucta* was 20 (Pl. XIV, fig. 1, 17; Text-fig. 1; 1). The chromosomes were bigger and a little wider than the chromosomes in both strains of *H. hirta*. In *H. aucta* the haploid number was 10 (counted by Mrs. E. Gluckmann, unpublished). In meiosis of *H. aucta* no abnormalities were observed and there is no difficulty in assuming a basic number of 10 in *H. aucta* as this is a rather frequent basic number in the Gramineae. We can easily see from the idiogram (see Text-fig. 2) of the diploid set of *H. aucta* that it consists of the following kinds of chromosomes:

- (a) One pair—the largest chromosomes—"V"-shaped with unequal arms and with two constrictions; one on the larger arm and the second on the tip of the "V" angle;
- (b) three "V"-shaped pairs with equal arms;
- (c) one "V"-shaped pair with unequal arms, a single median constriction and a very obtuse angle. This pair might as well be described as "L"-shaped;
- (d) three "L"-shaped pairs;
- (e) one "S"-shaped pair;
- (f) one short rod-shaped pair with median constriction.

A more detailed analysis of this idiogram shows three "V"-shaped pairs with equal arms consisting of different morphological pairs, having in common only the shape similar to a "V" and equality of the arms. The second pair from the left on the idiogram has two constrictions: one median (on top of the "V" angle, the kinetochore), and the second constriction on the arm. Also this pair is the largest amongst the "V"-shaped with the equal arms mentioned under (b).

The next pair on the right side is a much smaller one but is distinguished from the preceding pair not only by its size, but also by having only one median constriction. The next pair on the right (the fourth on the idiogram from the left side, text figure 2.) is smaller again, with the single median constriction. It is distinguished from the two pairs mentioned previously under (b) by its very obtuse angle. The three "L"-shaped pairs mentioned above under (d) also show variation in their characters. It is possible to distinguish fairly easily (1) one big "L"-shaped pair with the 2 constrictions, both on the longer arm; (2) two much smaller "L"-shaped pairs with the median constrictions.

Summing up the morphological features of the chromosome set in *H. aucta* we can say that it is easily recognisable not only by the number of 20, but also by the presence of such types of pairs as the "S"-shaped pair, the big "V"-shaped with unequal arms, and a single short rod-shaped pair with median constriction.

In *H. hirta* the author found two strains respectively with $2n = 30$ and $2n = 44$, growing sometimes very near each other.

The diploid set of $2n = 44$ may be described first (see the idiogram text fig. 2, Pl. XIV, fig. 18, 19, and Text-fig. 1; 2, 3, 4, in this connection).

Here we can recognise the following groups:

- (a) Seven "V"-shaped pairs with equal arms;
- (b) one "V"-shaped pair (N2) with unequal arms and two constrictions, one on the bigger arm and a median one;
- (c) one pair "S"-shaped (N9);
- (d) seven "L"-shaped pairs;
- (e) six rod-shaped pairs.

In describing in detail the morphology of the above set it will be noticed that amongst these "V"-shaped pairs with equal arms the following types will be found:

- (1) the largest "V"-shaped pair which has either two or sometimes even three constrictions. In the majority of metaphasic plates the constrictions were two in number with usually one on the bigger arm and one (probably the kinetochore) in the median position;
- (2) a smaller pair than the previous one (N3) with two constrictions on both arms;
- (3) two still smaller pairs (N4 and N5) with median constrictions but differing from each other in size;
- (4) three pairs, again smaller (N6, 7, 8) differing from the above described groups under (1) to (3) by their distinctly obtuse angle. For example, pair N8 could be described as a small "L"-shaped pair because the chromosomes have a very obtuse angle.

In the group of "L"-shaped chromosomes (mentioned under (d)) there are the following types:

- (1) four "L"-shaped pairs (N10, 11, 12, 15) differing markedly in size with two constrictions each. The secondary constriction is usually found on the bigger arm while the kinetochore usually has a median position. The pair N11 sometimes shows 2 constrictions on one homologous chromosome and one on the other. This is due, possibly, to insufficient differentiation;
- (2) three smaller "L"-shaped pairs (N13, 14, 16) have one median constriction. They differ less in size amongst themselves than those of the former group.

Under heading (e) six rod-shaped pairs have been listed. Amongst them we can distinguish two types:

- (1) three rod-shaped pairs with one subterminal constriction (N17, 20, 22); and
- (2) three rod-shaped pairs with one median constriction (N18, 19, 21).

The morphology of the last group was very difficult to determine due to the smallness of the chromosomes. The pairs number 20, 21 and 22 are the smallest of the set and it was sometimes difficult to decide whether the constriction was really subterminal or median.

The *H. hirta* strain with $2n = 30$ chromosomes has a diploid set consisting of the following groups:

- (a) five "V"-shaped pairs (N1, 4, 6, 7, 8) with equal arms;
- (b) one "V"-shaped pair with unequal arms and two constrictions;
- (c) six "L"-shaped pairs; and
- (d) three short rod-shaped pairs.

In comparing this set with the former strain ($2n = 44$) we can note without difficulty a striking similarity between the two sets. The $2n = 30$ strain simply has a set of $2n = 44$ strain chromosomes less seven definite pairs. These missing pairs are:

- (a) N3 and N5 pairs from "V"-shaped groups with equal arms;
- (b) the "S"-shaped pair;
- (c) the smallest N16 pair from the "L"-shaped group;
- (d) three pairs N18, 19, 21 are absent from the short rod group.

In other words, all the chromosomes in the above group which have median constrictions are absent, and all the short rod-shaped chromosomes with subterminal constrictions are present.

As a result of this study it was found easy to recognise roots of *H. hirta*, $2n = 30$ strain, without counting the chromosomes.

The metaphase plates were better spread and the chromosomes less crowded; the absence of the "S"-shaped pair (N9) was easily noted,

also the "V"-shaped pair with unequal arms; and finally the presence of only 3 short rod-shaped pairs—these are the leading distinctive features.

The "S"-shaped pair is such a characteristic feature that it deserves some additional remarks. Many dozens of metaphases of the strain with $2n = 30$ from 3 localities were investigated but the "S"-shaped pair was found only once (see Text-fig. 1; 3). In this metaphase it was not very distinctly expressed. The number of chromosomes was the same, 30, so the only explanation of its appearance was to suppose that it had developed from some pair of "L"-shaped chromosomes. As this occurred only in one single metaphase it could not be decided which pair of "L"-shaped chromosomes has this tendency of transformation into "S"-shaped chromosomes. Probably the "S" shape of this chromosome has a mechanical condition such that it lies in the metaphase plate more frequently with one of the short arms perpendicular to the plane of drawing. This would mean that it could be taken for a "L"-shaped or even for a rod-shaped chromosome. The single occurrence in the slides of the $2n = 30$ strain would probably represent a 1 per cent frequency or perhaps even less.

With this reservation the absence of "S"-shaped chromosomes in $2n = 30$ strain can still serve as a useful differential character, but it must be compared with the other two mentioned above, especially when only 3 short rod-shaped pairs are present.

It is rather difficult to define distinct morphological differences between the strains $2n = 30$ and $2n = 44$. The most distinct ones are perhaps: (1) a bluish colour of the leaves (as opposed to green in the strain $2n = 44$), (2) white nerves on the leaves (as opposed to green nerves in the $2n = 44$).

On the other hand it must be remembered that the chromosomic races and strains do not necessarily differ in morphological characters. The observation on *Bromus inermis* recently published by Hill and Meyers (5) in which there are many chromosomic but only two morphological strains is an illustration of such behaviour. An even more striking example is provided by *Poa pratensis* with numerous strains ranging from $2n = 28$ to $2n = \pm 124$. Amongst all these strains conspicuous morphological distinctions do not exist. They differ rather in physiological properties (Kramer, 17). A final illustration is provided by Meyers and Hill (23) whose estimation of the number of "intraspecific" chromosomic races in the Gramineae is as high as 99 (from this estimate *Poa pratensis* was excluded).

DISCUSSION.

Hyparrhenia aucta was included in cytological investigations of *Hyparrhenia hirta* mainly for the reason that this species commonly

grows nearby or mixed with *H. hirta*. (For the same reason a count of the chromosomes of *Schizachyrium semiberbe* (Text-fig. 1; 5) is included as well. In this species $2n = 50$ was found in adventitious root tips.) There is, however, no close karyological similarity between these two *Hyparrhenia* species.

Firstly, the chromosome numbers differ widely; secondly, the diploid chromosomes of *H. aucta* are longer and a little wider than those of *H. hirta*. (Compare Text-fig. 1; 1 with 1; 2, 3). (Note: The magnification of fig. 1 is $\pm 1,400\times$ and fig. 2 and 3 $\pm 2,800\times$.)

If one ignored these essential differences for a moment, one would find some similarities between the *H. hirta* strain $2n = 44$ and *H. aucta* in the following pairs of chromosomes:

- (1) The "S" chromosome pair N9 of *H. hirta* is similar to *H. aucta* N7;
- (2) the biggest "L" of *H. hirta* (N10) is comparable with *H. aucta* N6, with one important reservation, viz. that the distribution of 2 constrictions is not completely identical;
- (3) the single short rod pair with median constriction in *H. aucta* is similar to the corresponding pair of *H. hirta*, probably to N18. Moreover, there are some similarities between two pairs of "L"-shaped chromosomes with median constriction to pairs corresponding in height (N16 and 14) of *H. hirta*. Regarding this comparison with critical reserve it would appear that only the similarities between "S"- and the short rod-shaped chromosomes would stand, though they differ very much in size.

A distinct similarity in only two pairs is certainly not enough to establish affinity in morphology in complements of chromosomes.

Gluckmann confirmed the writer's finding of a haploid number of ten in *H. aucta* but did not observe any irregularity in meiosis of P.M.C. (unfortunately her drawings were not preserved).

These facts probably are sufficient to establish that *H. aucta* is a diploid species with the basic number ten. This would be in accord with the general estimation of basic chromosome numbers in Gramineae and especially in the tribe Andropogoneae. Avdulov (2, p. 82—83) expressed as his opinion that the basic numbers in Andropogoneae are 9 and 10. In his diagram of the frequency of basic numbers in the monocotyledons the basic number 10 has the highest frequency; it occupies the second place (Avdulov, p. 328) after the basic number of 12. In Darlington and Janaki-Ammal's Chromosome Atlas (6) this number is given for many Andropogoneae. The acceptance of 10 as a basic number for *H. aucta* will involve some inconvenience in regard to *H. hirta* chromosomal valency.

The existence of 2 forms in *H. hirta* in the Transvaal and on the Rand was known long ago to Prof. Phillips and his co-workers. It was difficult to define the morphological differences between the two strains as there are overlapping forms. For this reason both forms have been fixed from the inception of this work but unfortunately the fixed material of the $2n = 30$ strain, which was collected at the Frankenwald Experiment Station and which was provisionally described as "tall blue from Frankenwald", has been spoilt by accident in two consecutive years in an overheated thermostat. For this reason the first count concerned the strain $2n = 44$.

On account of this occurrence Garber's (11) publication achieved priority. The above author mainly described chromosome numbers of different species of *Sorghum* and only incidentally included *H. hirta* as a member of the same tribe of Andropogoneae by way of illustration. He investigated meiosis only and found the haploid number to be 15. His publication reached South Africa in 1947 and soon afterwards the count of $2n = 30$ in root tip metaphases of the "tall blue" strain was confirmed.

The American author was asked to send seed of his strain and to give any information about its origin. In due course information from Mr. B. P. Robinson was received, who kindly supplied a sample of seeds. From this correspondence it appeared that the strain of *H. hirta* was introduced to the U.S.A. from two institutions in the Union of South Africa in 1941, namely: (1) from the McGregor Museum, Kimberley; (2) from Frankenwald University Experimental Station. Mr. Robinson wrote that it was impossible to state from which of the two places the strain, which Garber and he himself investigated cytologically, originated. There was no evidence in the U.S.A. of differences registered between them. Thus far it has not been possible to obtain seed or further information about this strain from the McGregor Museum, Kimberley. Nevertheless, it has been thought reasonable to assume that the Kimberley strain also has a number $2n = 30$. If this is not true, there should be some evidence in American institutes of registered differences, or of the failure of germination of the seeds.

Soon afterwards good root tip material of both strains was obtained from the U.S.A. and from the "tall blue" form from Frankenwald. It appeared that both strains are karyologically identical, as the diploid number in both was the same ($2n = 30$). Morphologically both strains also were identical.

In a quick investigation of meiosis of the "tall blue" form no irregularities were found.

Another strain of *H. hirta*, investigated from two localities (1. Witwatersrand University; 2. Frankenwald Experiment Station) was, however, karyologically quite different. The root tip metaphases were very crowded; the chromosomes overlapped frequently and counting was extremely difficult. The diploid number consistently was 44 from all investigated localities (see the list of strains given later). There were few rare instances in which the number found was $44 + 1$ fragment.

This indistinct fragment was small, without any constriction. No relationship could be found between it and the other chromosomes. Moreover, during the investigations of meiotic metaphases and diakinesis in the anther (see former chapter) no body corresponding to the above fragment was found in spite of the great instability in the number of bivalents.

As to the nature and importance of the fragments we first may briefly consider the general position of this question in the world cytological literature where it has been discussed for about 30 years.

Broadly speaking, there are three types of supernumerary chromosomes and fragments: (1) The supernumerary B-chromosomes, described by Ostergren (33) and Lima de Faria (20), both in *Anthoxanthum*; (2) "Standard" fragments described in Rye by Müntzing (26). These are fragments of the size of the smallest normal chromosomes, in any case they are no bigger; (3) "iso-fragments" (Müntzing, 26), much smaller than the smallest of the normal chromosomes and usually smaller than the "standard fragments".

The B-chromosomes are usually heterochromatic and when present in some plants they appear during mitosis in every organ.

The "standard fragments" in rye are smaller than the smallest of normal chromosomes. They have distinct subterminal kinetochores. They are separated in meiosis and later exhibit non-disjunction, which means both daughter fragments go to one cell.

The "iso-fragments" are the smallest but they still have a distinct kinetochore and are easily recognised in rye by a delay in the separation of the chromatids, which have four free ends during mitotic metaphase. The iso-fragments have little genetical effect in rye when they are single or double, but in plants in which they accumulate in larger numbers they produce quite distinct negative effects (Müntzing, 25, Popoff, 34). In particular they are able to cause a decrease in fertility, as shown both in a reduced ability of seeds to germinate and in a smaller output of seeds. Müntzing (l.c.) has shown their deleterious effect on pollen grain germination. In view of the above remarks in connection with the rare fragments in *H. hirta* strain $2n = 44$ it is clear that the Hyparrhenia fragments do not fit the above types.

Firstly, they are never heterochromatic; secondly, they are deprived of a kinetochore; thirdly, they are not constant, and fourthly, their participation in mitosis has not been proved.

As to the origin of the supernumerary chromosomes and fragments there are two prevailing views. The first attempts to explain their appearance by duplication of one of the normal chromosomes or one of its arms, for example, *Zea mays* (Müntzing, 25).

The second view is based on the fragmentation of the normal chromosomes as an origin of fragments. This view has its extreme expression perhaps in Chakravartis' paper (5), where this author explains the origin of many new species, especially those with aneuploid numbers, in the families Cucurbitaceae, Musaceae, Zingiberaceae, Cannaceae, Scitamineae, by transverse fragmentation of normal chromosomes in place of secondary constriction. It is quite possible that the rare fragments in *H. hirta* have a similar origin. This can, however, be expressed only as pure conjecture as there is no evidence either of shortening of normal chromosomes or of direct observation of fragmentation.

The nature of *H. hirta* fragments and their origin are quite obscure, but it is not thought that they play any important part in the heredity of *Hyparrhenia*.

From the above it seems extremely improbable that *H. hirta* and *H. aucta* should have the same basic number, namely ten, and still more improbable is Garber's proposition of a basic number of five.

Thus far no distinct affinity between chromosomes of *H. aucta* and *H. hirta* has been found, which may be due to the fact that the plants have different basic numbers. We can reasonably assume a basic number of 10 for *H. aucta* and of 15 for both *H. hirta* strains. The basic number 15 is one of least frequent numbers in Monocotyledons (see Avdulov, p. 398, and his discussion of frequency of chromosome numbers). It has a fourth place in rarity, after "4" as the rarest number, and 17, 18. On the other hand, its frequency is about the same as the basic number 5 proposed by Garber (l.c.). We have already examples in Gramineae of a basic chromosome number of 15 in the tribes Paniceae (Pennisetum $2n = 45$), Oryzae (Darlington and Janaki-Ammal, p. 325) and in Stipeae (l.c. p. 350—351). There are further not less than 7 species with $2n = 44$. For the latter the author proposes a basic number of 11. This, however, is not acceptable for *H. hirta* for the following reasons: Firstly, it would not explain the diploid number 30 in the strain "tall, blue" from Frankenwald of the same species; secondly, it would mean that the $2n = 44$ strain of *H. hirta* is tetraploid. The low fertility in the above strain and the frequent variation in numbers of bivalents in meiosis do not support this

presumption. Apparently, there is no agreement on the question of the most frequent basic chromosome number in Gramineae. Wanscher (44) considers the most frequent basic number in this family to be $n = 7$, which is present in 89 species. Next in order of frequency comes $n = 14$ with 76 species, $n = 10$ in 10 species and $n = 5$ is present only in 1 species.

The tribe Andropogoneae according to Nielson (30, p. 370—371) definitely has a prevailing basic number $n = 10$ with few exceptions, but $n = 5$ is not even considered at all by this author.

In the writer's opinion the only reasonable choice is to assume 15 as a basic number, at least temporarily until such time as present inadequate knowledge of the chromosomes of the genus *Hyparrhenia* has been enriched by further investigations. On the basis of this assumption we can come to two further conclusions, viz.: (1) In the genus *Hyparrhenia* there are probably two lines of development; one with a basic number of 10 and the other with a basic number 15; (2) in *H. hirta* there are two strains; one diploid, with high fertility and $2n = 30$ and one triploid monosomic, much less fertile, with $2n = 44$.

The weights of mature seeds give some support to the above assumption. It has been generally stated that the seeds of polyploids are heavier than the seeds of diploids. Thus it was noted on the 16th of June, 1944, that the average weight of 100 seeds of the $2n = 30$ strain was 60 mgm. and of 100 seeds of the $2n = 44$ strain 75 mgm. The mature seeds for this test had been collected 2 months earlier and selected by hand using a magnifier. The increase in weight represents a difference of 20 per cent. In spite of great variations in weight over the seasons a one-year estimation could serve as a useful illustration of the polyploid nature of the $2n = 44$ strain.

The figures for germinability of the seed also fit in well with the above assumption. In germination experiments with the *H. hirta* strain $2n = 44$ rather low percentages were obtained. The highest for many years was 54 per cent, though the usual rate lay between 30 per cent to 40 per cent and very often was less than 30 per cent. The germination capacity of the *H. hirta* strain $2n = 30$ in the U.S.A. is much higher. As stated by Robinson and Potts (37) it is as high as 85 per cent and the average is 75 per cent. Germination tests of the pure seeds of $2n = 30$ strain from Frankenwald never gave results as high as the foregoing. Following on some observations on the time of opening of florets and shedding of pollen grains the following tentative explanation of this behaviour is put forward: Both strains of *H. hirta* are growing at Frankenwald in close proximity. The flowers open and shed pollen about the same time and thus the chances of inter-strain pollination equal those of intra-strain pollination.

These two kinds of pollen grains are completely different in their chromosomic content and fertilisation value. Practically all pollen grains

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of the strain $2n = 30$ have 15 chromosomes and are highly compatible in fertilisation, while of the pollen grains coming from the $2n = 44$ strain none has a compatible number of chromosomes. Nearly half of them have $n = 22$, and then follow the numbers 24, 18, 16 and 14. All these are even numbers with few exceptions. To be compatible for the female gamete in the plant with $2n = 30$, the foreign pollen grain should at least have an odd number.

Rich co-pollination with the pollen grains shed by $2n = 44$ plants with the 15 chromosome type is probably the main cause of the lower fertility of this strain under local conditions as compared with high percentage germination of the same strain in the U.S.A.

The strain of *H. hirta* $2n = 30$ introduced from South Africa to the U.S.A. found itself there artificially isolated from "contamination" by related pollen, as there are no indigenous *Hyparrhenia* species in the U.S.A. Both strains introduced were apparently of the same chromosomic value ($2n = 30$) and were therefore pollinated only with pure pollen grains containing $n = 15$ chromosomes. This then is the chief cause of a much higher percentage of germination and higher fertility.

In connection with the above assumption it would be of interest to know something more definite about the geographical distribution of both strains in the Union of South Africa. Unfortunately, the writer can make only a very modest contribution to this question.

Strains from the following localities were the only ones investigated:—

- | | |
|---|-----------|
| 1. From the Experimental Station, Rietvlei, near Irene, | |
| Transvaal | $2n = 44$ |
| 2. Grootfontein Experiment Station, Middelburg, Cape.. | $2n = 30$ |
| 3. Experiment Station, Dohne | $2n = 44$ |
| 4. From Jaegerbush, Losberg | $2n = 44$ |
| 5. From Jaegerbush, Losberg, 1 plant only | $2n = 30$ |
| 6. From the Frankenwald Experiment Station | $2n = 30$ |
| 7. From the Frankenwald Experiment Station | $2n = 44$ |
| 8. Witwatersrand University, Milner Park, Johannesburg | $2n = 44$ |

In addition to the above list of localities there is reasonable evidence to suppose that the Kimberley strain sent in 1941 to the U.S.A. had probably $2n = 30$ chromosomes. (See above for the reasons for this supposition.)

The above findings are certainly not sufficient for making any assumptions about the geographical distribution. It is not even known which strains are predominant in the various localities, but it could be supposed that the samples were sent to the author on the ground that one type of *Hyparrhenia* appeared to differ from the common type or because it was dominant in the region.

In view of this scarcity of information the author feels it would be wise to limit his statements only to the conditions prevailing in the Transvaal, especially around Johannesburg, where both strains grow practically in the same habitats. The $2n = 44$ strain of *H. hirta* is much more frequent, while the *H. hirta* $2n = 30$ strain prevails rather on old vleis.

In addition to the above some details of the technique of counting should perhaps be added. From each locality not less than 10 roots usually belonging to 10 different plants were investigated. In this way each statement on chromosome numbers from different localities is based on not less than 10 slides, with numerous metaphases. The roots of *H. hirta* $2n = 44$ from the University and Frankenwald and the strain $2n = 30$, the "tall blue", from Frankenwald and that from the U.S.A. were exceptions to this rule, as with different methods of squashing, staining and fixation more than 100 roots of the strain $2n = 44$ and 30 to 40 roots of the $2n = 30$ strain were studied.

In spite of these numbers the amazing uniformity, with one single exception, was striking. Only in the plants from Jaegerbush, Losberg, a metaphase with $2n = 56$ was found on one occasion. This root belonged to the plant with $2n = 44$ chromosomes. Unfortunately, from one crowded metaphase nothing could be learnt about the morphology of the chromosomes. That was probably a case of a mutant root.

Mention has already been made of the crowdedness of metaphases in the *H. hirta* strain $2n = 44$ and the difficulty of counting them. In order to spread the chromosomes better and make the counting easier experiments were undertaken with pre-treatment of the roots with cold treatment before fixation (see Darlington and La Cour (7), Delaunay, 9). There was also another reason for trying this method. In the course of previous years there was some evidence in cytological literature that cold pre-treatment would reveal a third kind of constriction in chromosomes (Resende and Cabral, 35), which would be constant for given species and given chromosomes. One of the investigators—F. Resende—even proposed to give them the special term "Olistherozones" (Nature, v. 144, p. 481 and Acta Portugaliae Biologica, Vol. 1, fasc. 2, 1945).

For these two reasons methodical pre-treatment at a temperature of 4°C . was given to root tips before fixation. The roots were fixed at intervals of one hour starting with a treatment of one hour and increasing up to 24 hours. The shortening and spreading of metaphasic chromosomes was at a maximum after 4—6 hours pre-treatment. However, the added advantage of better spreading and shortening of chromosomes was conspicuously reduced in value by the peculiarity that they were now distributed in many planes, sometimes very distant ones. The danger of omission in the counting of some chromosomes occurring in different

planes is obvious. With longer pre-treatment the chromosomes became crowded, and with still longer treatment sticking occurred, while after 24 hours the metaphases were shrunk, exhibiting indistinct clumps of chromatin with frequent bridges in anaphases.

With regard to the new tertiary constrictions, the so-called "Olistherozones", the results of our experiments were completely negative. This is in line with previous experiments on *Senecio isatideus* (Krupko and Goldsmith, 13). In this instance constrictions which were visible without pre-treatment disappeared completely. This was not the case, however, with *H. hirta*. The kinetochores and secondary constrictions are clearly seen when maximum spreading has been achieved, i.e., after 4—6 hours of treatment, but new constrictions were not revealed.

The temperature of 4°C. was chosen for these experiments because Delaunay claimed that he obtained the best results at that temperature with the majority of plants. It is quite possible that *H. hirta* would respond differently at different temperatures.

For the same purpose soaking in cold water before fixation was also tried. This gave some improvement in the spreading of chromosomes after 2—3 hours of treatment (see also Goldsmith and Krupko, 13) which was just the reverse of Gluckmann's results with *Themeda* (unpublished).

Considering the *H. hirta* strain $2n = 44$ as a monosomic triploid plant we could expect that the majority of chromosomes, in somatic metaphases at least, would be found not in homologous pairs but in homologous triplets. In order to check this aspect of the karyogram an attempt was made many times to arrange the drawings of morphologically similar chromosomes, cut out of the paper in triplets, but this invariably failed. Only 6 rod-shaped pairs could sometimes be arranged in triplets, but this was without significance because of the smallness of the rod shaped chromosomes. Also there was great uncertainty in determining the nature of the constrictions in these chromosomes. It has been pointed out that it was often difficult to decide which type of constriction was present, namely, subterminal or median. It is usually easy to arrange all other chromosomes in pairs.

It is supposed that this aspect of the karyogram has some theoretical significance with regard to the character of triploidy. It probably means that triploidy in this strain was achieved very long ago. The *H. hirta* strain $2n = 44$ is an old triploid species in which triplets have had enough time to adapt themselves to the diploid physiological function, and become pairs. It is possible that in early age this strain was a simple triploid with a full complement of 45 chromosomes. For some reason unknown at present one chromosome of the complement was gradually excluded

in disjunction, and the strain finally became a monosomic triploid. If this is so the fragment mentioned previously which occurs sometimes in root metaphases may be the relic of the odd chromosome.

ADDITIONAL OBSERVATIONS.

During this work on *Hyparrhenia hirta* some additional observations were made not directly connected with the cytology of the plant, but probably being of some interest in the expansion of our knowledge of South African grasses.

First may be mentioned observations on the dichogamy of *Hyparrhenia* florets, which were carried out on the *H. hirta* strain from the Grootfontein College of Agriculture which has $2n = 30$ as described above. The spikelets and florets were marked with coloured wool threads. In this way the observation was soon made that there is no protandric behaviour at all, contrary to the prevailing statements of German ecologists in many textbooks of Ecology. Nearly all of them state that protandry is the prevailing condition in the Gramineae family (in Europe). Apparently it is not the case in *H. hirta*, at least in the strain from Grootfontein. In this strain protogyny is the prevailing condition. The stigmas appeared early in the morning, sometimes between 8—10 hours. They first had a white colour which was maintained during the first day, but the next day changed to a light yellow. On the second morning the stigmas were coloured a deep red and this colour was maintained, gradually changing to violet-red and ultimately to black-red. Quite possibly this change of colour is connected with pollination or even with the fertilisation process.

Similar observations of protogyny were made by Artschwaeger and Maguire (1) in *Sorghum vulgare*, though they did not mention the colour of the stigmas in the plants. A remarkable fact is that the florets remain open for the very short time of 30—60 minutes. In addition to the protogynic behaviour, cases of simultaneous appearance of stigmas and anthers were noted in *H. hirta*.

The second observation is connected with the number of xylem strands in the primary roots. In *H. hirta* the vascular bundles are all pentarch, without any exception. It may be of value to compare the statements on the same subject in current anatomical literature. Mostly this item is ignored, or there are only observations on cereals, from which a general idea of the structures of other grasses are drawn. For example, Avery (3) mentioned that the primary roots of *Triticum* are hepta-octarch and according to this author maize roots have even more "rays".

The third observation concerns the structure of growing points of adventitious roots. In *H. hirta* all four histogens are present (see Pl. XIV, fig. 15, Pl. XV, fig. 21). It exhibits a classical picture of the Monocot

structure: the calyptragen gives rise to the calyptra, the dermatogen to the epidermis, the periblem to the cortex, and the plerome to the central cylinder. As said before, there is a tendency amongst grass growers to see the structure of cereals as a model for all grass structures. In this case such a generalisation would be completely wrong. According to Percival Nelson (29), Hector (14) and Standford (40) the cereals have only three histogens, cortex and epidermis having one common histogen for both tissues. In this respect again *H. hirta* differs from the cereals.

Avdulov (2, pp. 324—325) attached special attention to the shape and to the position of the first leaf in the grasses. In his opinion Hackel's (1889) statement that a lanceolate or elliptic first leaf in the Andropogoneae with a more or less horizontal position is a primitive character as compared with linear or awl-shaped leaves, as prevailing amongst other grasses, should be extended to the other tribes. He found (l.c., p. 325) the same shape and character of the first leaf in all 6 tribes of Series A in the Gramineae (according to Hackel's system), which he calls the subfamily Sacchariferae. Moreover, when this shape occurs in other species of Series B of the family it is invariably connected with primitiveness of characters or with the relic nature of that species. In the light of the above, Avdulov's interpretation is useful perhaps in giving a picture (Pl. XV, fig. 24) of the first leaf of *H. hirta*. It is distinctly elliptical and it keeps a practically horizontal position, at least in the beginning. Its margin is ciliated. Unfortunately, the photograph included fails completely to reproduce ciliation of the margin, which however can easily be seen with a $\times 10$ magnifier. In this respect the first leaf of *H. hirta* agrees with Hackel's rule for the tribe Andropogoneae and with Avdulov's extension of that rule to the subfamily Sacchariferae.

The fifth observation is connected with the great difficulty experienced in squashing fresh root tip sections of *H. hirta* for quick staining and counting of chromosomes. Schlerenchymic tissues in root tips were sought in vain but very soon it was apparent that resistance to the squashing force is due to the thick external wall of the epidermis (see Pl. XV, fig. 22). The thickness of this wall is frequently equal to the diameter of the lumen of the epidermis cells and sometimes it is twice as thick as the diameter. This external wall stains distinctly with 1 per cent Congo Red, revealing its cellulosic nature. The same wall may be stained pink with ruthenium red, the colour appearing evenly on the whole thickness of the wall without striations or layers appearing. The impression was gained that the external layer of the wall is a solid cellulosic membrane. Below this wall there is probably a dense elastic layer of cellulosic and pectic slime, highly resistant to pressure. This extremely thick wall covers only the meri-

stematic region and the more differentiated parts of the root have an epidermis with a wall of solid cellulose of usual thickness.

RECOMMENDATIONS.

Having in mind the ecological importance of the genus *Hyparrhenia* in Africa and the possibility of the use on a wide scale of some of its species in soil conservation measures, the author regards it as very important that the following lines of investigation will be carried out:

(1) Counting of chromosome numbers and analysis of meiosis of all species of *Hyparrhenia* in Africa; (2) chromosome counting and chromosome morphology of *H. hirta* strains from different countries (up to the Red Sea); (3) collection of facts on the geographical distribution of chromosomal strains of *H. hirta* in the Union of South Africa, based on direct counting of chromosomes in plants from different localities, but not based on the external morphological characters; (4) experimental plots of the diploid strain of *H. hirta* should be arranged in different parts of the country with strict isolation of the plots from pollination by other strains of *H. hirta* or by other *Hyparrhenia* species.

It is the writer's hope that these plots would prove the greater fertility of the diploid strain in isolated conditions.

SUMMARY.

There are probably at least two lines of development in the genus *Hyparrhenia*: one with a basic chromosome number = 10, and the other with a basic number = 15.

Hyparrhenia aucta has the following chromosome numbers: $2n = 20$; $n = 10$. Its basic number is probably 10.

Hyparrhenia hirta has two strains, diploid with $2n = 30$, $n = 15$, monosomic triploid with $2n = 44$. The latter has various numbers of bivalents in pollen grain mother cell meiosis: 22, 20, 18, 16, 14.

Laggers are frequent and persisting up to the 1-nucleated stage of the young pollen grain.

The basic number of chromosomes in *H. hirta* is probably 15.

Of the mature pollen grains of the above-mentioned strain ± 20 per cent is degenerated. Normal looking pollen grains germinate very rarely and with difficulty. The tubes are short, growing slowly and are short lived.

There are some mature pollen grains with supernumerary nuclei.

The above disturbances in pollen grain development and the unbalanced number of chromosomes in male gametes are probably the chief causes of low fertility of the monosomic triploid strain.

Schizochyrium semiberbe has $2n = 50$.

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PLATE XIV.

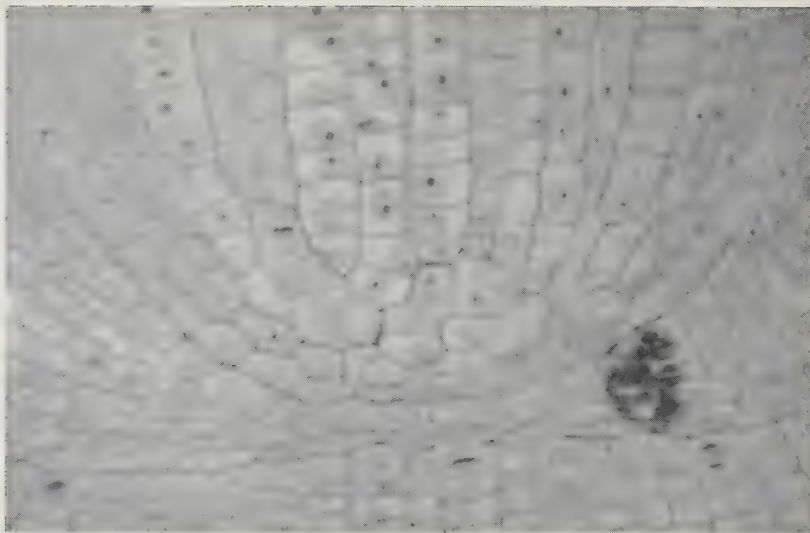
- FIG. 1 AND FIG. 17.—*H. aucta*, root tip metaphase— $2n = 20$. Fix. Levitsky (5:5) with 4% formol. Stained—Iron haematox. Thickness— $7\ \mu$. Photo. taken with B. & L. apochr. $\times 90$ oil immers. and Comp. oc. $\times 10$. Yellow filter. Magnification $\pm 1150\times$.
- FIGS. 2 to 7, 6 to 11, 16.—*H. hirta*, strain $2n = 44$. P.g.m.c. Fix.—Carnoy. Stained—Iron haematox. Thickness $8\ \mu$. Taken with B. & L. apochromat. $\times 90$ oil immers. and Comp. oc. $\times 12$. Magnification $\pm 1000\times$.
- FIGS. 12 to 14.—*H. hirta*. Similar strain. Smears, fixed in Nawashin, stained with Crystal Violet after Newton. Mature p. grain.
- FIG. 1.—Metaphase, $2n = 20$.
- FIG. 2.—P.g.m.c. diakinesis with $n = 14$. Taken in two levels. For continuation see Fig. No. 3. Seen 5 bivalents and nucleolus. Taken with green filter No. 58 Wratten. Exposure $17''$.
- FIG. 3.—The same pollen grain m.c. another level. Here is visible continuation of nucleolus and of one bivalent indistinct above it. Beneath the nucleolus two bivalents (not seen in Fig. 2). All together one can see 9 bivalents not seen in Fig. 2.
- FIG. 4.—P.g.m.c. 1st meiotic metaphase with $2n = 22$. Taken with green filter No. 58 Wratten. Exposure $20''$.
- FIG. 5.—P.g.m.c. 1st meiotic metaphase with $n = 18$. Filter the same. Exposure $25''$.
- FIG. 6.—P.g.m.c. 1st meiotic anaphase with numerous laggings. Green filter. Exposure $15''$.
- FIG. 7.—P.g.m.c. 1st meiotic middle telophase with 4 laggings. Taken without filter. Exposure $4''$.
- FIG. 8.—*H. hirta*, strain $2n = 44$. Acetocarmine squash. P.g.m.c. 1st meiotic telophase. Laggings. Taken with green filter No. 58 Wratten. Exposure $23''$. Optics and magn. like above.
- FIG. 9.—Old tetrads with the laggings. Taken without filter. Exposure $4''$.
- FIG. 10.—V.y.p.gr. (gonies), 1-lagger. Taken without filter. Exposure $10''$.
- FIG. 11.—The same. Dwarf nucleus—dw. n. nucleus—n. without filter. Exposure $3''$.
- FIG. 12.—Mature p. gr. with 4 nuclei, taken in 2 levels; for continuation see Fig. 13. Yellow filter. Exposure $22''$.
- FIG. 13.—Continuation of Fig. 12. Exposure $5''$.
- FIG. 14.—Mature p.g. with 5 nuclei; two of them indistinct—slide fading. Yellow and green filters. Exposure $35''$.
- FIG. 15.—*H. hirta*, strain $2n = 30$, from Frankenwald. Fix.—Nawashin. Stained—Haematox. + Fast green. Thickness $8\ \mu$. Root tip—longitudinal, showing 4 histogens. Taken with B. & L. obj. $\times 20$ Comp. oc. $\times 10$. Magnification $\pm 250\times$.
- FIG. 16.—V.y.p.g. (gonies). Four laggings. Without filter. Exposure— $10''$.
- FIG. 17.—Like Fig. No. 1. Metaphase in root tips epidermis with numerous "V"-shaped chromosomes and "S"-shaped one.
- FIG. 18.—*H. hirta*, strain $2n = 30$. Root tip metaphase. Fixation Nawashin. Staining—Crystal Violet. Thickness $10\ \mu$. B. & L. Fl. oil immers. $\times 98$, Comp. oc. $\times 10$. Taken in two levels. Exposure: upper photo $8''$, lower one $16''$. Magnification $\pm 1200\times$.
- FIG. 19.—*H. hirta*, strain $2n = 44$. Root tip metaphase. Fix.—Levitsky (5:5) with 10% formol. 1% Chrom. acid—3 days. Staining—iron haematox. Thickness $7\ \mu$. Optics as in Fig. 18. Exposure $16''$. Magnification $\pm 1200\times$.
- FIG. 20.—*H. hirta*, strain $2n = 44$, root tip, metaphase. Fix. and staining as in Fig. 18. B. & L. oil immers. $\times 97$. Comp. oc. $\times 7.5$. Final magnification $\pm 3300\times$.

PLATE XV.

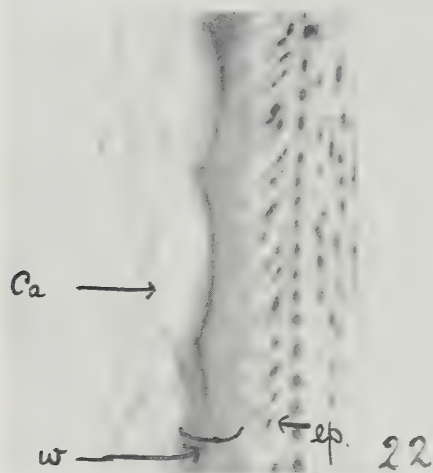
- FIG. 21.—*H. hirta*, strain $2n = 30$ from Frankenwald. Root tip longitudinal, showing growing point, 4 histogens. Fix.—Nawashin. Staining—iron haematox + fast green. Thickness $8\ \mu$. Taken with B. & L. obj. $\times 20$. oc. $\times 20$, enlarged ± 4 times. Total magnification $\pm 1000\times$.
- FIG. 22.—*H. hirta*, adv. roots. Growing point longitudinal sect. showing unusually thick ext. wall of epidermis cells. Stained: Congo red + haematox. Thickness $8\ \mu$. Taken with B. & L. obj. 60, ocul. $\times 10$; exposure $2''$. Magnification ± 500 . ep. = epidermis cell, w = external wall, ca = calyptra cells.
- FIG. 23.—*H. hirta*, strain $2n = 44$. Enlargement of Fig. 19, Pl. XIV $\pm 3300\times$, to show more distinctly the shape of chromosomes.
- FIG. 24.—*H. hirta*, strain $2n = 44$. Seedlings.



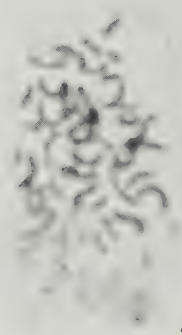
PLATE XIV.
Karyology of *Hyparrhenia*.



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PLATE XV.
Karyology of *Hyparrhenia*.